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EXAMINER	
THOMAS, DAVID C	
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SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	12/22/2006	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/797,333	PINTER ET AL.	
	Examiner	Art Unit	
	David C. Thomas	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 October 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-111,119-125 and 140-146* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-111,119-125 and 140-146* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *26 January 2005*. June 17, 2004
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election without traverse of Group I, claims 1-111, 119-125, and 140-146, in the reply filed on October 12, 2006 is acknowledged. Claims 112-118, 126-139, and 147-152 have been canceled.

Claim Rejections - 35 USC § 102

2. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

3. Claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144 are rejected under 35 U.S.C. 102(b) as being anticipated by Chenchik et al. (U.S. Patent No. 5,759,822).

Chenchik teaches a method of preparing a DNA molecule (amplification of DNA fragments by PCR, column 4, lines 53-55), comprising:

obtaining at least one DNA molecule (from both natural and synthetic sources, column 12, lines 18-19);

randomly fragmenting the DNA molecule to produce DNA fragments (by random shearing of the DNA, column 11, lines 62-65);

modifying the ends of the DNA fragments to provide attachable ends (such as by treatment with terminal transferase, column 7, lines 27-33 and column 9, lines 3-6);

attaching an adaptor having at least one known sequence and a nonblocked 3' end to the ends of the modified DNA fragments to produce adaptor-linked fragments,

wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and a 5' end of the adaptor (attachment of adapters that lack 5-phosphate on lower oligomer results in only upper oligomer of adapter being ligated since the upper oligomer is not blocked at 3' end to allow ligation to 5' end of fragment, while a nick would result between 3' end of fragment and 5' end of lower oligomer, column 8, lines 19-20 and column 7, lines 27-31);

extending the 3' end of the modified DNA from the nick site (nick would be extended resulting in displacement of lower oligomer by polymerase in order to fill in ends and eliminate any single-strandedness after attachment and ligation of upper oligomer of addapter that has a 5'-blocked lower oligomer that cannot be ligated, column 4, lines 62-62 and column 8, lines 19-20); and

amplifying a plurality of the adaptor-linked fragments (amplification of fragments with unique adapters at each end serving as priming sites, column 5, lines 8-14).

With regard to claim 2, Chenchik teaches a method wherein said at least one DNA molecule is further defined as genomic DNA (DNA can be from natural sources including viruses, bacteria, yeast, plants, insects and animals, column 12, lines 18-22).

With regard to claim 3, Chenchik teaches a method wherein said modifying step is further defined as modifying the ends of the DNA fragments to comprise blunt double stranded ends (fragments can be prepared such that adapters are ligated to blunt ends, column 7, line 27-31).

With regard to claim 4, Chenchik teaches a method wherein said modifying step is further defined as modifying the ends of the DNA fragments to comprise an overhang of at least 1 nucleotide (ends can be prepared such that adapters are ligated to sticky ends or ends containing oligo (dA) tailing, column 7, line 27-33 and column 9, lines 3-6).

With regard to claims 5 and 6, Chenchik teaches a method wherein said randomly fragmenting the DNA molecule comprises mechanical fragmentation such as hydrodynamic shearing, column 11, lines 62-65).

With regard to claim 13, Chenchik teaches a method wherein the modifying step comprises repair of at least one 3' end of the DNA fragment (3' ends of fragments are extended to fill in single-stranded regions after annealing of adapters, column 4, lines 62-64).

With regard to claim 14, Chenchik teaches a method wherein the modifying step comprises subjecting said DNA fragment to 3' exonuclease activity, 5'-3' polymerase activity, or both (fragments can be treated with a DNA polymerase to extend 3' end during attachment of adapters, column 7, lines 27-36).

With regard to claim 38, Chenchik teaches a method wherein said modifying step and said attaching step occurs concomitantly (ends can be modified by attachment of adapters directly to blunt ended fragments, column 7, lines 27-31).

With regard to claim 47, Chenchik teaches a method wherein said attaching step is further defined as subjecting said DNA fragments to a blunt end adaptor, a 5' overhang adaptor, a 3' overhang adaptor, or a mixture thereof (adapters are attached with a 5' overhang at one end and blunt end at the other, column 8, lines 3-14).

With regard to claim 48, Chenchik teaches a method wherein said adaptor comprises at least one of the following features: absence of a 5' phosphate group (column 8, lines 20-21); a 5' overhang (column 8, lines 3-6); and a blocked 3' base (3' amine to prevent extension, column 8, lines 27-32).

With regard to claim 49, Chenchik teaches a method wherein said 5" overhang comprises about 5 to about 100 bases (5' overhang of several adapters is greater than 5 bases, see Table 1).

With regard to claims 50, 52, and 53, Chenchik teaches a method wherein said attaching is by ligating the adaptor to the DNA fragment by enzymatic ligation using T4 DNA ligase (ligation is performed by T4 DNA ligase, column 8, lines 12-13).

With regard to claim 56, Chenchik teaches a method wherein said adaptor comprises a sequence of 5 "-CCCTT-3 " (primer TPA 2 begins with CCCTT and is thus complementary to an adapter and would be incorporated into fragment during PCR as an adapter, Table 1, seq ID. No. 22, and column 9, lines 7-8).

With regard to claim 57, Chenchik teaches a method wherein the DNA fragments are blunt ended and a 3' adenine is added to the blunt ended DNA fragments by polymerase (blunt end fragments can be tailed with oligo (A) with terminal transferase, column 7, lines 27-31 and column 9, lines 3-7).

With regard to claim 58, Chenchik teaches a method wherein the adaptor comprises a first primer and a second primer, said first primer greater in length than said second primer (Type 1 adapters are partially double-stranded with one long and one short oligomer, column 8, lines 3-6).

With regard to claims 59 and 61, Chenchik teaches a method wherein the second primer comprises a blocked 3' end (3' amine can be placed on shorter oligomer to prevent extension, column 8, lines 28-32).

With regard to claim 60, Chenchik teaches a method wherein the adaptor comprises at least one blunt end (adapter can have equal-length upper and lower oligomers and can be ligated onto blunt ends, column 8, lines 11-14).

With regard to claims 62 and 63, Chenchik teaches a method wherein the adaptor comprises one oligonucleotide having two regions complementary to each other to form a double-stranded region of said adaptor that is blunt-ended, said regions separated by a linker region (such as formation of pan-like structure when adapters of complementary sequence are at either end of a single-stranded fragment, column 5, lines 21-23, column 7, lines 59-65 and Figure 1).

With regard to claim 66, Chenchik teaches a method wherein said extending step comprises subjecting the adaptor-linked fragments comprising the nick to a mixture comprising:

DNA polymerase (KlenTaq/Pfu DNA polymerase, column 21, line 25);

deoxynucleotide triphosphates (column 21, line 24); and

suitable buffer, under conditions wherein polymerization occurs from the 3' hydroxyl of the nick (amplification during PCR displaces one strand of adapter, column 21, lines 20-22 and Table 3) and .

With regard to claims 67 and 68, Chenchik teaches a method wherein the method further comprises heating the mixture (sample is heated to 75°C to inactivate ligase, column 21, lines 10-11).

With regard to claims 69-71, Chenchik teaches a method wherein the polymerase is a strand-displacing polymerase such as *Taq* polymerase (KlenTaq/Pfu DNA polymerase, column 21, line 25).

With regard to claim 73, Chenchik teaches a method wherein said amplifying step comprises polymerase chain reaction, said reaction utilizing a primer complementary to a sequence of the adaptor (PCR performed utilizing primers that hybridize to adapters, column 21, lines 58-61 and Table 3).

With regard to claim 77, Chenchik teaches a method wherein said at least one DNA molecule is comprised in a cell (DNA can be obtained from natural sources such as bacteria, plants, insects, and animals, column 12, lines 18-21).

With regard to claim 78, Chenchik teaches a method wherein said at least one DNA molecule is not comprised in a cell (DNA can be synthetic or from a virus, column 12, lines 18-21).

With regard to claim 80, Chenchik teaches a method wherein said obtaining method is further defined as obtaining the at least one DNA molecule from blood (peripheral blood leucocytes, column 3, lines 48-55).

With regard to claims 82 and 83, Chenchik teaches a method wherein said genomic DNA comprises bacterial genomic DNA, viral genomic DNA, fungal genomic

DNA, plant genomic DNA, or mammalian genomic DNA (DNA can be obtained from existing bacteria, virues, yeast, plants, insects, and animals, column 12, lines 18-21).

With regard to claim 84, Chenchik teaches a method wherein said at least one DNA molecule comprises a portion of a genome (fragments of genomic DNA can be used, column 15, lines 30-34).

With regard to claims 89 and 90, Chenchik teaches a method of preparing a DNA molecule, comprising: obtaining a plurality of DNA molecules, said DNA molecules defined as fragments from at least one larger DNA molecule (fragments can be obtained from genomic DNA, column 15, lines 30-33);

modifying the ends of the DNA fragments to provide attachable ends (such as by treatment with terminal transferase, column 7, lines 27-33 and column 9, lines 3-6);

attaching an adaptor having at least one known sequence and a nonblocked 3' end to the ends of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and a 5' end of the adaptor (attachment of adapter containing primer binding site to the fragments and filling in ends to eliminate any single-strandedness, or directly ligating to the sticky or blunt ended DNA, column 4, lines 62-62 and column 7, lines 27-31);

extending the 3' end of the modified DNA from the nick site (nick can be extended by polymerase or by ligation of adapter to fragment, column 4, lines 62-62 and column 7, lines 27-31); and

amplifying a plurality of the adaptor-linked fragments (amplification of fragments with unique adapters at each end serving as priming sites, column 5, lines 8-14).

With regard to claim 91, Chenchik teaches a method of amplifying a genome, comprising the steps of;

obtaining at least one DNA molecule (from both natural and synthetic sources, column 12, lines 18-19);

randomly fragmenting the DNA molecule to produce DNA fragments (by random shearing of the DNA, column 11, lines 62-65);

modifying the ends of the DNA fragments to provide attachable ends (such as by treatment with terminal transferase, column 7, lines 27-33 and column 9, lines 3-6);

attaching an adaptor having at least one known sequence and a nonblocked 3' end to the ends of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and a 5' end of the adaptor (attachment of adapter containing primer binding site to the fragments and filling in ends to eliminate any single-strandedness, or directly ligating to the sticky or blunt ended DNA, column 4, lines 62-62 and column 7, lines 27-31);

extending the 3' end of the modified DNA from the nick site (nick can be extended by polymerase or by ligation of adapter to fragment, column 4, lines 62-62 and column 7, lines 27-31); and

amplifying a plurality of the adaptor-linked fragments (amplification of fragments with unique adapters at each end serving as priming sites, column 5, lines 8-14).

With regard to claim 92, Chenchik teaches a method of generating a library, comprising the steps of:

obtaining at least one DNA molecule (from both natural and synthetic sources, column 12, lines 18-19);

randomly fragmenting the DNA molecule to produce DNA fragments (by random shearing of the DNA, column 11, lines 62-65);

modifying the ends of the DNA fragments to provide attachable ends (such as by treatment with terminal transferase, column 7, lines 27-33 and column 9, lines 3-6);

attaching an adaptor having at least one known sequence and a nonblocked 3' end to the ends of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and a 5' end of the adaptor (attachment of adapter containing primer binding site to the fragments and filling in ends to eliminate any single-strandedness, or directly ligating to the sticky or blunt ended DNA, column 4, lines 62-62 and column 7, lines 27-31);

extending the 3' end of the modified DNA from the nick site (nick can be extended by polymerase or by ligation of adapter to fragment, column 4, lines 62-62 and column 7, lines 27-31).

With regard to claim 93, Chenchik teaches a method wherein said method further comprises amplifying a plurality of the adaptor-linked fragments (amplification of plurality of fragments with unique adapters at each end serving as priming sites, column 5, lines 8-14).

With regard to claim 100, Chenchik teaches a method wherein said DNA molecule is further defined as a genome (from both natural sources such as bacteria, virus, or from animal cells, column 12, lines 18-19).

With regard to claim 102, Chenchik teaches a method wherein said ligase is T4 DNA ligase (ligase is T4 DNA ligase, column 8, lines 13-14).

With regard to claim 103, Chenchik teaches a method wherein said adaptor is a blunt end adaptor or a 5' overhang adaptor (adapters are attached with a 5' overhang at one end and blunt end at the other, column 8, lines 3-14).

With regard to claim 104, Chenchik teaches a method wherein the adaptor comprises a first primer and a second primer, said first primer greater in length than said second primer (Type 1 adapters are partially double-stranded with one long and one short oligomer, column 8, lines 3-6).

With regard to claim 105, Chenchik teaches a method wherein said first primer lacks a 5' phosphate (upper primer lacks 5' phosphate, column 8, lines 20-21).

With regard to claim 106, Chenchik teaches a method wherein the buffer comprises a divalent cation, a salt, adenosine triphosphate, dithiothreitol, or a mixture thereof (buffer comprises $MgCl_2$, DTT, and ATP, column 22, lines 29-31).

With regard to claims 107 and 108, Chenchik teaches a method wherein the conditions comprise a large molar excess of linkers to DNA fragment ends (adapter are present at 5 μM, in great excess over fragment, column 22, lines 26-31) .

With regard to claim 109, Chenchik teaches a method wherein said method further comprises amplifying the DNA fragments using a primer complementary to the

adaptor (PCR was performed with primers complementary to adapters, column 22, line 63 to column 23, line 6).

With regard to claim 119, Chenchik teaches a method of amplifying at least one RNA molecule, comprising the steps of:

obtaining at least one RNA molecule (human placental RNA, column 19, lines 26-34);

reverse transcribing said RNA molecule to produce a cDNA molecule (conversion of RNA to cDNA, column 19, lines 35-36);

randomly fragmenting the cDNA molecule to produce DNA fragments (gene-specific primers were used to make cDNA, so fragmentation was not necessary, column 19, lines 41-46; random shearing could be used if preparing total cDNA, column 11, lines 62-65);

modifying the ends of the DNA fragments to provide attachable ends (such as by treatment with terminal transferase, column 7, lines 27-33 and column 9, lines 3-6);

attaching an adaptor having at least one known sequence and a nonblocked 3' end to the ends of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and a 5' end of the adaptor (attachment of adapter containing primer binding site to the fragments and filling in ends to eliminate any single-strandedness, or directly ligating to the sticky or blunt ended DNA, column 4, lines 62-62 and column 7, lines 27-31);

extending the 3' end of the modified DNA from the nick site (nick can be extended by polymerase or by ligation of adapter to fragment, column 4, lines 62-62 and column 7, lines 27-31); and

amplifying a plurality of the adaptor-linked fragments (amplification of fragments with unique adapters at each end serving as priming sites, column 5, lines 8-14).

With regard to claim 120, Chenchik teaches a method of amplifying a population of DNA molecules comprised in a plurality of populations of DNA molecules, said method comprising the steps of:

obtaining a plurality of populations of DNA molecules, wherein at least one population in said plurality comprises DNA molecules having in a 5' to 3' orientation the following (plurality of fragments were produced by restriction digestion of human genomic DNA, and T7 adapter was added, column 22, lines 14-33):

a known identification sequence specific for said population (tissue plasminogen activator (TPA) sequence is present in population and specific to primer, column 22, lines 4-9); and

a known primer amplification sequence (TPA 1 primer is added to mix, column 22, lines 63-67); and

amplifying said population of DNA molecules by polymerase chain reaction, said reaction utilizing a primer for said identification sequence (specific population is amplified using sequence-specific primers, column 22, line 63 to column 23, line 6).

With regard to claim 121, Chenchik teaches a method wherein said obtaining step is further defined as:

obtaining a population of DNA molecules, said molecules comprising a known primer amplification sequence (plurality of fragments, including TPA sequence, were produced by restriction digestion of human genomic DNA, and T7 adapter was added, column 22, lines 14-33);

amplifying said DNA molecules with a primer having in a 5' to 3' orientation the following:

the known identification sequence (tissue plasminogen activator (TPA) sequence is present in population and specific to primer, column 22, lines 4-9); and

the known primer amplification sequence (TPA1 primer is added to mix, column 22, lines 63-67); and

mixing said population with at least one other population of DNA molecules (by amplification with second sequence-specific primer, TPA2, to generate second population of overlapping fragments, column 23, lines 7-18).

With regard to claim 122, Chenchik teaches a method wherein said population of DNA molecules is a genome (DNA can be genomic from bacteria, plants, yeast, or animals, column 12, lines 18-22).

With regard to claim 123, Chenchik teaches a method of amplifying a population of DNA molecules comprised in a plurality of populations of DNA molecules, said method comprising the steps of:

obtaining a plurality of populations of DNA molecules, wherein at least one population in said plurality comprises DNA molecules having in a 5' to 3' orientation the

following (plurality of fragments were produced by restriction digestion of human genomic DNA, and T7 adapter was added, column 22, lines 14-33):

a known identification sequence specific for said population (tissue plasminogen activator (TPA) sequence is present in population and specific to primer, column 22, lines 4-9); and

a known primer amplification sequence (TPA 1 primer is added to mix, column 22, lines 63-67); and

isolating said population through binding of at least part of the single stranded known identification sequence of a plurality of said DNA molecules to a surface (defined polynucleotides can be immobilized at pre-defined regions on a solid support matrix, column 14, lines 28-30); and

amplifying the isolated DNA molecules by polymerase chain reaction, said reaction utilizing a primer for said primer amplification sequence (specific population is amplified using sequence-specific primers, column 22, line 63 to column 23, line 6).

With regard to claim 125, Chenchik teaches a method wherein said isolating step is further defined as binding at least part of the single stranded known identification sequence to an immobilized oligonucleotide comprising a region complementary to the known identification sequence (defined polynucleotides can be immobilized at pre-defined regions on a solid support matrix complementary to polynucleotide sequence, column 14, lines 28-30).

With regard to claim 140, Chenchik teaches a method of preparing a DNA molecule, comprising:

obtaining a population of DNA molecules having ligatable ends of unknown nature (from both natural and synthetic sources, column 12, lines 18-19; fragments can be prepared by random shearing of the DNA, some of which will blunt ended and some staggered, column 11, lines 62-65);

providing to said population one or more known forms of adaptors, wherein said adaptors each comprise at least one known sequence and at least one oligonucleotide having a 3' extendable end (attachment of adapters of known sequence that lack 5-phosphate on lower oligomer that results in only upper oligomer of adapter being ligated since the upper oligomer is not blocked at 3' end to allow ligation to 5' end of fragment, column 8, lines 19-20 and column 7, lines 27-31);

determining ligatability of said one or more known forms of adaptors to said DNA molecules (adaptors of that ligate to either blunt or sticky-ends can be designed for optimal ligation, column 8, lines 13-18 and column 9, line 19 to column 10, line 3); and

ligating said known one or more forms of adaptors to said DNA molecule (using ligase such as T4 DNA ligase, column 8, lines 13-14).

With regard to claim 141, Chenchik teaches a method wherein said determining step is further defined as identifying a ratio of ligatable forms of adaptors corresponding to the nature of the ends of the DNA molecules in the population, and wherein said ligating step is further defined as introducing to said population a plurality of said adaptors in said ratio (adaptors can have sticky-ends or blunt ends and can also have 5'-phosphates added, or 3' amine groups to prevent extension, column 8, lines 13-32).

With regard to claim 142, Chenchik teaches a method wherein said ligatability of said one or more forms of adaptors are determined separately (blunt-end and sticky-end adaptors can be tested separately, column 8, lines 13-18).

With regard to claim 143, Chenchik teaches a method wherein said method further comprises the step of extending the 3' end of said oligonucleotide by polymerization to produce an extended product (attachment of adapters that lack 5-phosphate on lower oligomer results in only upper oligomer of adapter being ligated since the upper oligomer is not blocked at 3' end to allow ligation to 5' end of fragment, while an extendable nick would result between 3' end of fragment and 5' end of lower oligomer, column 8, lines 19-20 and column 7, lines 27-31; such extension would result in removing any remaining single-strandedness and filling to the ends of each fragment containing an adaptor, column 4, lines 62-66).

With regard to claim 144, Chenchik teaches a method wherein said method further comprises the step of amplifying said extended product by polymerase chain reaction (fragments are subjected to PCR amplification, with unique adapters at each end serving as priming sites, column 5, lines 8-14).

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 7, 8, 12, 19-28, and 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Teasdale (U.S. Patent No. 5,759,821).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144.

Chenchik does not teach a method wherein said randomly fragmenting the DNA molecule comprises a chemical fragmentation method such as heating. Chenchik also does not teach a method wherein modifying step is further defined as subjecting said ssDNA molecules to a plurality of random primers of 5-9 bases in length and DNA polymerase activity.

With regard to claims 7, 8, and 12, Teasdale teaches a method wherein said randomly fragmenting the DNA molecule comprises a chemical fragmentation method such as heating (genomic DNA is cleaved to a minimum size by chemical means such as heating, column 3, lines 21-39 and column 4, lines 43-50).

With regard to claim 19, Teasdale teaches a method wherein said DNA fragments comprise a plurality of ssDNA molecules (after fragmentation of DNA, ssDNA is generated by initial heating step of PCR, column 3, lines 13-17) and said modifying step is further defined as subjecting said ssDNA molecules to a plurality of random primers and DNA polymerase activity, under conditions wherein said blunt double stranded fragments are thereby generated (amplification of fragments using random primers which would generate blunt-ended products, which can then used for attaching primers, column 2, lines 39-51).

With regard to claim 20, Teasdale teaches a method wherein the random primers further comprise a known sequence at their 5' end (some primers are used which have specific known sequence to yield a characteristic set of bands from a particular set of sites randomly disperse over the genome, column 1, lines 49-52).

With regard to claims 22-26, Teasdale teaches a method wherein the random primers are pentamers, hexamers, septamers, octamers, or nonamers (random primers are about 10 bases in length, column 2, lines 42-44).

With regard to claim 34, Teasdale teaches a method wherein said polymerase comprises nick translation activity (treatment of DNA fragments with Taq polymerase is performed during amplification with random primers, column 3, lines 1-7).

Teasdale does not teach a method of producing adaptor-linked fragments wherein a nick remains on one strand of the duplex between the 3' end of the fragment and the 5' end of the adaptor. Teasdale also does not teach a method of treating the single-stranded ends of fragments using a 3' exonuclease activity and wherein at least

one ssDNA molecule comprises a blocked 3' end. Teasdale also does not teach a method wherein the random primers are phosphorylated at the 5' end or comprised of at least one base analog.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and amplifying DNA fragments randomly generated by techniques such as shearing and those of Teasdale which also teach methods of preparing sample DNA for downstream processes such as amplification and sequencing but using a chemical method such as heating in a salt solution and using random primers to generate PCR products. Thus, an ordinary practitioner would have been motivated to combine the methods of Teasdale and Chenchik for preparation of random fragments since the method of heating taught by Teasdale provides an alternative general method of fragmentation that can be coupled with amplification using random primers of about 10 bases in length to produce blunt-ended fragments that can then be used in the methods of Chenchik to add adaptors for subsequent attachment of adaptors of known sequence.

7. Claims 9, 10, 15-18, 29-33, 35, 40-46, 72, and 74 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Sagner et al. (U.S. Patent No. 5,714,318).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144. Chenchik also teaches the use of exonuclease III treatment to modify the ends of DNA fragments or PCR products (column 9, lines 8-10).

Chenchik does not teach a method wherein randomly fragmenting the DNA molecule comprises enzymatic fragmentation such as digestion with DNAse I or in the presence of Mg^{2+}. Chenchik also does not teach a method of treating DNA fragments to modify their ends using Klenow or T4 DNA polymerases. Chenchik also does not teach a method of labeling probes or primers with labeled nucleotides or extending nicks with labeled nucleotides.

With regard to claims 9 and 10, Sagner teaches a method wherein said randomly fragmenting the DNA molecule comprises enzymatic fragmentation such as digestion with DNAse I (column 3, lines 29-34).

With regard to claims 15, 16, 17, 18, 29, 35, 40-43, 45, and 46, Sagner teaches a method wherein both of said 3' exonuclease activity and said 5'-3' polymerase activity are comprised in the same enzyme such as is the case with Klenow or T4 DNA polymerase (column 3, lines 25-34 and column 9, lines 22-25).

With regard to claims 30-33, Sagner teaches a method wherein the polymerase used to modify the ends of DNA fragments is a non-strand-displacing polymerase such as T4 DNA polymerase or a strand-displacing enzyme such as Klenow (column 3, lines 25-34 and column 9, lines 22-25).

With regard to claim 44, Sagner teaches a method wherein said enzymatic fragmentation occurs in the presence of Mg^{2+} and said modifying step is further defined as subjecting said DNA fragments to random primers, 5'-3' polymerase activity and 3'-5' exonuclease activity (DNA can be fragmented with restriction endonucleases which require Mg^{2+} and treated with T4 or Klenow polymerases, column 3, lines 29-37).

With regard to claims 72 and 74, Sagner teaches a method wherein at least one deoxynucleotide triphosphate is labeled (probes can be prepared incorporation of labeled nucleotides during synthesis, column 6, lines 47-59).

Sagner does not teach a method of producing adaptor-linked fragments wherein a nick remains on one strand of the duplex between the 3' end of the fragment and the 5' end of the adaptor. Sagner also does not teach a method of treating the ends of fragments using the 3' exonuclease activity of Exonuclease III.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and amplifying DNA fragments randomly generated by techniques such as shearing and those of Sagner which also teach methods of preparing sample DNA for downstream processes such as sequencing but also teach, in addition to shearing, enzymatic methods of fragment preparation, and also teach the use of a variety of additional enzymatic methods to polishing fragment ends in preparation of attachment of adaptors. Thus, an ordinary practitioner would have been motivated to combine the methods of Chenchik and Sagner for preparation of random fragments since the method of enzymatic treatment with DNase I taught by Sagner provides an alternative general method of fragmentation that can be coupled with the method of attachment of adaptors and post-treatment taught by Chenchik to make the ends fully duplex, which ensures the amplification using adaptor-specific primers will be efficient for both strands. The methods of Sagner using a variety of enzymes to polish the ends is essential to produce

blunt-ends when using random methods of fragmentation such as shearing or DNase I treatment, and allows efficient and consistent ligation of the adaptors to all fragments.

8. Claims 9, 11 and 101 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Drmanac (U.S. Patent No. 6,309,824).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144.

Chenchik does not teach a method wherein randomly fragmenting the DNA molecule comprises enzymatic fragmentation such as Cvi JI restriction enzyme digestion.

Drmanac teaches a method of enzymatic fragmentation using the two-base restriction endonuclease CviJI that generates random, relatively small fragments (column 18, line 62 to column 19, line 4).

Drmanac does not teach a method of further modifying the ends of the fragments, attaching adaptors, and amplifying the adaptor-linked fragments.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and amplifying DNA fragments randomly generated by techniques such as shearing and those of Drmanac which also teach methods of preparing sample DNA for downstream processes such as sequencing but using a two-base recognition restriction endonuclease, CviJI, to generate highly random fragments comparable to other methods of random fragmentation. Thus, an ordinary practitioner would have been

motivated to use the method of Drmanac for rapid preparation of random fragments for subsequent attachment of adaptors since the use of CviJI restriction endonuclease produces blunt end fragments that are readily ligatable (Drmanac, column 19, lines 5-18), and require lesser amounts of DNA and fewer steps than most other methods, without the need for end repair (Drmanac, column 19, lines 19-26).

9. Claims 36, 37, 75, and 76 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Jung et al. (J. Clin. Pathol. (2002) 55:55-57).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144.

Chenchik does not teach a method wherein said modifying step occurs in the presence of additives known to facilitate polymerization through GC-rich DNA, such as 7-Deaza-dGTP.

With regard to claims 36, 37, 75, and 76, Jung teaches a method of performing PCR and sequencing reactions in the presence of 7-Deaza-dGTP in order to generate full-length products from templates that are GC-rich, such as those containing CpG islands (p. 55, column 1, line 29 to column 2, line 6).

Jung does not teach a method of producing adaptor-linked fragments wherein a nick remains on one strand of the duplex between the 3' end of the fragment and the 5' end of the adaptor.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and

amplifying DNA fragments randomly generated by techniques such as shearing, followed by enzymatic treatment to modify the ends in preparation for attachment of adaptors and subsequent amplification and those of Jung which teaches methods of synthesizing DNA using modified bases which facilitate synthesis through GC rich regions such as CpG islands in processes such as DNA sequencing or PCR. Thus, an ordinary practitioner would have been motivated to combine the methods of Chenchik and Jung since the use of modified bases such 7-Deaza-dGTP allows generation of full-length products in targets comprising GC rich regions (Jung, p. 55, column 1, line 29 to column 2, line 3. Furthermore, the addition of 7-Deaza-dGTP can be helpful when working with low amounts of DNA template of poor quality (Jung, p. 55, column 2, lines 4-6. Thus, DNA synthesis of any regions near the ends of fragments rich in GC content would benefit from the use of this modified nucleotide when modifying the fragment either prior to or after attachment of the adaptor.

10. Claim 39 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Campbell et al. (J. Biol. Chem. (1980) 255:3726-3735).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144. Chenchik also teaches a method wherein said modifying step step is further defined as subjecting said DNA fragments to 3' exonuclease activity (treatment with exonuclease III, column 9, lines 8-10) or 5'-3' polymerase activity (addition of tails with terminal transferase, column 7, lines 27-33 and column 9, lines 3-6).

Chenchik does not teach a method wherein said enzymatic fragmentation occurs in the presence of Mn^{2+}.

With regard to claim 39, Campbell teaches a method of DNase I digestion of SV40 DNA in the presence of 0.66-10 mM Mn^{2+} (Table I and p. 3728, column 2, lines 36-47).

Campbell does not teach a method of further modifying the ends of the fragments, attaching adaptors, extending nicks and gap filling, and amplifying the adaptor-linked fragments.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and amplifying DNA fragments randomly generated by techniques such as shearing and those of Campbell which teach methods of preparing random DNA fragments using DNase I treatment. In addition, Campbell teaches methods of treatment with DNase I in the presence of Mn^{2+}, which leads to cleavage of predominantly double strands (Campbell, p. 3728, column 2, lines 42-47). Thus, an ordinary practitioner would have been motivated to combine the methods of Chenchik and Campbell for preparation of random fragments since the method of enzymatic treatment with DNase I using Mn^{2+} taught by Campbell provides an alternative general method of fragmentation that can be coupled with the method of attachment of adaptors and post-treatment taught by Chenchik to make the ends fully duplex, which ensures the amplification using adaptor-specific primers will be efficient for both strands. The methods of Campbell using DNase I using Mn^{2+} will generate larger amounts of cleaved duplex fragments

compared to using this enzyme in the presence of magnesium ions and will thus allow efficient and consistent ligation of the adaptors to fragments representing a greater portion of the original DNA sample.

11. Claims 51, 54, and 55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Wolffe et al. (U.S. Patent No. 6,511,808).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144.

Chenchik does not teach a method wherein said ligation is by chemical ligation or by enzymatic ligation using topoisomerase I wherein said adaptor is covalently attached to topoisomerase I at a 3' thymidine overhang or a blunt end.

With regard to claims 51, 54 and 55, Wolffe teaches a method of ligation of DNA fragments, including those that are blunt-ended, into a desired vector by topoisomerase-mediated enzymatic ligation or by chemical ligation (column 28, lines 40-50).

Wolffe does not teach a method of producing adaptor-linked fragments wherein a nick remains on one strand of the duplex between the 3' end of the fragment and the 5' end of the adaptor, and amplifying a plurality of the adaptor-linked fragments.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and amplifying DNA randomly generated fragments with adaptors attached to the termini and the methods of Wolffe for ligation of adaptors to blunt ends by enzymatic or chemical approaches. Thus, an ordinary practitioner would have been motivated to

combine the methods of Chenchik and Wolffe since Wolffe teaches alternate methods of ligation such as using a topoisomerase or chemical ligation, which may be more efficient than T4 ligase for ligation to blunt-ended fragments. Since most methods of random fragmentation will result in fragments containing blunt ends after polishing, it is likely that a number of ligation methods will need to be optizmized for any given DNA sample, since blunt-ended ligation is known to be less efficient than when using staggered ends generated by sequence-specific fragmentation by restriction endonucleases.

12. Claims 64, 65 and 124 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Kozerski et al. (Nucleic Acids Res. (2001) 29:1132-1143).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144.

With regard to claim 124, Chenchik also teaches a method wherein said obtaining step is further defined as:

obtaining a population of DNA molecules, said molecules comprising a known primer amplification sequence (plurality of fragments, including TPA sequence, were produced by restriction digestion of human genomic DNA, and T7 adapter was added, column 22, lines 14-33);

amplifying said DNA molecules with a primer comprising in a 5' to 3' orientation the following:

the known identification sequence (tissue plasminogen activator (TPA) sequence is present in population and specific to primer, column 22, lines 4-9);

the known primer amplification sequence (TPA1 primer is added to mix, column 22, lines 63-67); and

mixing said population with at least one other population of DNA molecules (by amplification with second sequence-specific primer, TPA2, to generate second population of overlapping fragments, column 23, lines 7-18).

Chenchik does not teach a method wherein a linker region in the primer or adaptor comprises a non-replicable organic chain of about 1 to about 50 atoms in length wherein said non-replicable organic chain is hexa ethylene glycole (HEG).

Kozerski teaches a nicked double-stranded oligonucleotide containing a non-nucleotide bridge comprised of hexaethylene glycol, which renders the oligonucleotide resistant to exonuclease cleavage (Abstract and p. 1132, column 2, lines 18-23).

Kozerski does not teach a method of producing adaptor-linked fragments wherein a nick remains on one strand of the duplex between the 3' end of the fragment and the 5' end of the adaptor, and amplifying a plurality of the adaptor-linked fragments.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and amplifying DNA randomly generated fragments with adaptors attached to the termini and the methods of Kozerski for design of oligonucleotides containing linker regions comprised of non-replicable organic chains such as hexaethylene glycol since the use of such chains in adaptors renders them inert to enzymatic processing (Kozerski, p.

1132, column 2, lines 18-22). Thus, an ordinary practitioner would have been motivated to combine the methods of Chenchik and Kozerski since Kozerski teaches the use of alternate methods of designing oligonucleotides that could be used as adaptors that are refractory to enzymatic processing such as exonuclease treatment or templating DNA synthesis. Such adaptors could be used to define the termini of adaptor-linked fragments since nick-translation or exonuclease degradation would proceed only to the linker region. Since these adaptors would also be resistant to degradation or serving as a template, it allows one to control enzymatic processing of selected strands within an adaptor sequence by modification of one of the two strands.

13. Claims 79 and 81 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Vodenicharov et al. (Cancer Letters (1996) 106:51-58).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144.

Chenchik does not teach a method wherein the at least one DNA molecule is cell-free fetal DNA in maternal blood or is cell-free cancer DNA in blood or wherein said physically isolated chromatin is isolated by centrifugation, electrophoresis, micro-filtration, affinity capture, or a combination thereof.

With regard to claims 79 and 81, Vodenicharov teaches a method of preparation of chromatin from mouse erythroleukemia cells using a series of centrifugation steps to isolate chromatin fragments (Abstract, p. 52, column 1, lines 23-24 and column 1, line 43 to column 2, line 22).

Vodenicharov does not teach a method of producing adaptor-linked fragments wherein a nick remains on one strand of the duplex between the 3' end of the fragment and the 5' end of the adaptor, and amplifying a plurality of the adaptor-linked fragments.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and amplifying DNA randomly generated fragments with adaptors attached to the termini and the methods of Vodenicharov for methods of preparation and isolation of chromatin from sources such as cancer cells, since the use of such isolation techniques produces stable fragments and can be applied to isolation of any chromatin material from any cellular source where stability of the material is essential, such as the methods of Chenchik where genomic DNA is further fragmented in a random manner. Thus, an ordinary practitioner would have been motivated to combine the methods of Chenchik and Vodenicharov since Vodenicharov teaches methods of isolation of chromatin by centrifugation which can be used to generate random fragments for subsequent attachment of adaptors. It is important that such a technique provide largely intact genetic material since the basis of the methods of Chenchik is to generate a representative random set of DNA fragments for attachment of adaptors and eventual amplification.

14. Claims 85-88 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Hubank et al. (Nucleic Acids Res. (1994) 22:5640-5648).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144.

With regard to claims 85-87, Chenchik also teaches a method wherein said adaptor is further defined as a first adaptor having a first known sequence and further comprises a homopolymeric sequence (type 2 adaptors contain a homopolymeric sequence in the suppressor portion of the adaptor and the primer becomes incorporated as an adaptor during PCR, column 9, lines 1-8; adaptor-fragments with unique adapters at each end serving as priming sites for amplification, column 5, lines 8-14; the PCR products can be digested to remove one of the strands of the adaptor, column 9, lines 8-10).

With regard to claim 88, Chenchik teaches a method of preparing a DNA molecule, comprising:

obtaining at least one DNA molecule (from both natural and synthetic sources, column 12, lines 18-19);

attaching a first adaptor having a first known sequence, a homopolymeric sequence and a nonblocked 3' end to the ends of the DNA molecule to produce first adaptor-linked molecules, wherein the 5' end of the DNA molecule is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA molecule and a 5' end of the adaptor (attachment of adapters having homopolymeric sequences in the suppressor portion, column 9, lines 1-3, that lack 5-phosphate on lower oligomer results in only upper oligomer of adapter being ligated since the upper oligomer is not blocked at 3' end to allow ligation to 5' end of fragment,

while a nick would result between 3' end of fragment and 5' end of lower oligomer, column 8, lines 19-20 and column 7, lines 27-31); and

amplifying a plurality of the adaptor-linked fragments (amplification of fragments with unique adapters at each end serving as priming sites, column 5, lines 8-14).

Chenchik does not teach a method of digesting the amplified adaptor-linked fragments to produce fragmented adaptor-linked fragments and attaching a second adaptor having a second known sequence to the ends of the fragmented adaptor-linked fragments to produce second adaptor-linked fragments.

With regard to claims 85-88, Hubank teaches a method of digesting the amplified adaptor-linked fragments to produce fragmented adaptor-linked fragments (adaptors can be digested with a restriction endonuclease to change adaptors on a cDNA fragment to form a driver DNA population, p. 5642, column 1, lines 16-18) and attaching a second adaptor having a second known sequence to the ends of the fragmented adaptor-linked fragments to produce second adaptor-linked fragments (second adaptors can be added after digestion to form tester DNA population, p. 5642, column 1, lines 18-23).

Hubank does not teach a method of producing adaptor-linked fragments wherein a nick remains on one strand of the duplex between the 3' end of the fragment and the 5' end of the adaptor, and treatment to extend the nick to remove single-stranded regions from the fragments.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and

amplifying DNA randomly generated fragments with adaptors attached to the termini and the methods of Hubank for methods of attaching second adaptor sequences to DNA fragments since second adaptors can be useful for applications such as preparation of a tester fragment population when performing representational difference analysis of cDNA (Hubank, p. 5642, column 1, lines 18-23), while digestion of fragments containing first adaptors can be used to produce driver populations (Hubank, p. 5642, column 1, lines 16-18). Thus, an ordinary practitioner would have been motivated to combine the methods of Chenchik and Hubank since Hubank teaches methods of digesting adaptor-linked fragments and adding second adaptors, which provides a convenient method for changing adaptors on a set of fragments into another adaptor sequence that can then be amplified by primers specific for the new adaptors.

15. Claims 94-99, 110, 111, 145, and 146 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chenchik et al. (U.S. Patent No. 5,759,822) in view of Keith et al. (U.S. Patent No. 5,093,245).

Chenchik teaches the limitations of claims 1-6, 13, 14, 38, 47-50, 52, 53, 56-63, 66-71, 73, 77, 78, 80, 82-84, 89-93, 100, 102-109, 119-123, 125, and 140-144.

With regard to claims 94-99, 110, and 111, Chenchik also teaches a method of preparing at least one DNA molecule or generating a library of DNA molecules, comprising:

admixing together:

a ligase (T4 DNA ligase, column 8, lines 13-14);

an adaptor (adaptor ligated with ligase to DNA fragment, column 8, lines 13-14); and

a buffer, under conditions wherein a plurality of the ends are ligated to said adaptor (adapters were ligated to digested DNA, column 22, lines 23-33).

Chenchik does not teach a method of preparing a DNA molecule by admixing an endonuclease, a ligase, an adaptor and a buffer under conditions wherein said DNA molecule is cleaved by said endonuclease to generate a plurality of DNA fragments, a plurality of the ends of which are ligated to said adaptor, wherein the method consists essentially of one step wherein the cleavage and ligation occur concomitantly under the same conditions without changing the buffer after the cleavage step. Chenchik also does not teach a method wherein said population of DNA molecules are obtained from serum or plasma.

With regard to claims 94-99, 110, and 111, Keith teaches a method of simultaneously fragmenting a DNA sample with a restriction endonuclease and ligating oligonucleotide sequences to the termini to provide primer sequences for amplification or other applications such as labeling (column 2, lines 29-51 and column 3, lines 25-35). The reaction mixture contains target DNA, T4 DNA ligase, a restriction endonuclease, oligonucleotides for attachment, and a buffer such that the cleavage and ligation occur concomitantly under the same conditions without changing the buffer after the cleavage step, without a precipitation step (Example 1, column 7, lines 17-36).

With regard to claims 145 and 146, Keith teaches a method wherein said population of DNA molecules is obtained from serum or plasma (source of sample may

be obtained from physiological media such as blood, serum, or plasma, column 3, lines 53-57).

Keith does not teach a method of producing adaptor-linked fragments wherein a nick remains on one strand of the duplex between the 3' end of the fragment and the 5' end of the adaptor, and treatment to extend the nick to remove single-stranded regions from the fragments.

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Chenchik for preparing and amplifying DNA randomly generated fragments with adaptors attached to the termini and the methods of Keith for ligation of adaptors to fragments generated during the ligation reaction in the same buffer since this method allows a streamlined one-step method for generating adapter-linked fragments and also helps prevent unintended ligation of the fragments to each other since an excess of adaptor sequences is present during the reaction (Keith, column 2, lines 35-39 and Example 1, column 7, lines 17-36). Thus, an ordinary practitioner would have been motivated to combine the methods of Chenchik and Keith since Keith teaches alternate methods of ligation such as performing the ligation concomitantly with an endonuclease digestion step, which allows a greater number of fragments to be linked to an adaptor instead of religating to each other. The one-step method is also simpler and faster, making it more suitable for high-throughput assays when preparing large numbers of samples.

Conclusion

16. Claims 1-111, 119-125, and 140-146 are rejected. No claims are allowable.

Correspondence

17. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David C. Thomas whose telephone number is 571-272-3320. The examiner can normally be reached on 5 days, 9-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

David C. Thomas 12/20/06
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